Chemical Engineering Research and Design 201 (2024) 169–175

Contents lists available at ScienceDirect

Chemical Engineering Research and Design

journal homepage: www.elsevier.com/locate/cherd

Green chemistry approach for stereoselective aldol condensation catalyzed by amino acids under microflow conditions

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ARTICLE INFO

Keywords:
Microfluidic synthesis
Aldol reaction
Asymmetric organocatalysis
Amino acids

ABSTRACT

Over the recent years, the pharmaceutical field has been under significant pressure to adapt to the principles of green chemistry. The industry is facing the challenge of environmental protection and securing people's health while sustaining its fundamental goals of efficiency and selectivity optimization. In this work, the reaction processes optimization has been acheived through the microfluidic approach to the widely used aldol condensation reaction. In accordance with the green chemistry principles, we have found that the best conditions include aqueous ethanol solvent and easily available amino acids used as catalysts. Asymmetric aldol condensation of *para*-nitrobenzaldehyde with cyclohexanone catalyzed by L-*tert*-Leu under microfluidic conditions provided the product with high yield and diastereoselectivity (anti/syn = 28:1) and 98% enantiomeric excess. The microfluidic reactor process, compared to the classical batch method (in a flask), provides comparable product yield, while the reaction time was reduced by an order of magnitude. It should be noted that this approach allows to carry out the reactions at low (below zero) temperatures with greater enantioselectivity.

1. Introduction

The competitive modern pharmaceutical industry is engaged in a permanent search for new breakthrough technologies (Rubin et al., 2006; Domokos et al., 2021; Yadav et al., 2022). Microfluidic methods provide possibilities for significantly accelerating the processes and carrying them out under mild conditions (Nunes and Stone, 2022; Oshchepkov et al., 2020a). These methods increase conversion and selectivity, shorten reaction times and reduce the impact of side reactions (Zeng et al., 2023). This is important for synthetic organic chemistry and the chemical industry (Fukuyama et al., 2008; Oshchepkov et al., 2020b; de Almeida et al., 2019). Microfluidics allows continuous reactions to proceed in capillary or microchannels, in contrast with traditional batch reactors (Convery and Gadegaard, 2019). Significant advances in the area of flow chemistry using continuous production methods over the past decade have led to the improvements in the production of chiral biologically active substances on an industrial scale that stays in compliance with the environment protection requirements (Plutschack et al., 2017; Hughes, 2020).

Technological processes design and optimization become especially challenging for technical chemistry when the objective includes obtaining optically pure substances (Rachwalski et al., 2013). Recently, this task has been successfully addressed through the development and implementation of microfluidic technologies (Kochetkov et al., 2022). Continuous asymmetric catalysis is especially promising in this regard, since the attractiveness of these technologies for the multifunctional chiral compounds production includes the considerations of "green" chemistry (Hernández and Juaristi, 2012). The stereoselective aldol condensation has a special place among such processes. It leads to a widely used class of chiral β-hydroxyketones (Osborne, 2004; Snider, 2005), which makes this reaction one of the most effective methods for carbon-carbon bond formation in modern organic synthesis.

Aldol condensation of cyclohexanone (**1**) with *para*-nitrobenzaldehyde (**2**) in the presence of chiral catalysts results in a mixture of four stereoisomers of 2-(hydroxy(4-nitrophenyl)methyl)cyclohexanone (**3**) At high temperatures, b-hydroxyketone dehydrates to form (E)− 2-(4-nitrobenzylidene)cyclohexan-1-one (**4**). This reaction was suitable to investigate the effect of the catalyst characteristics and the

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https://doi.org/10.1016/j.cherd.2023.11.055
Received 23 September 2023; Received in revised form 17 November 2023; Accepted 25 November 2023
Available online 28 November 2023

reaction conditions on the stereoselectivity under classical setup (Lin et al., 2009; Zheng et al., 2017; Guillena et al., 2008; Li et al., 2009; Juaristi, 2021; Samanta et al., 2005; Guizzetti et al., 2006; Bañón-Caballero et al., 2013; Qian et al., 2010; Agarwal and Peddinti, 2011; Xu et al., 2009; Kochetkov et al., 2011, 2012; Mitsui and Parquette, 2009). (Fig. 1).

The reaction had a high yield (up to 96%), good diastereoselectivity (*anti / syn* = 9: 1), and high enantioselectivity but proceeded extremely slow, over several days (Lin et al., 2009; Zheng et al., 2017; Guillena et al., 2008; Li et al., 2009; Juaristi, 2021; Samanta et al., 2005; Guizzetti et al., 2006; Bañón-Caballero et al., 2013; Qian et al., 2010; Agarwal and Peddinti, 2011; Xu et al., 2009; Kochetkov et al., 2011, 2012; Mitsui and Parquette, 2009). Free amino acids, proline mimetics, and small peptides used as catalysts in some cases provided high stereoselectivity, but the reactiuons still took considerable amount of time to achieve high conversion rates (Córdova et al., 2006; Cho and Kim, 2014; Jiang et al., 2010; Karmakar et al., 2011). Acceleration was observed only in a ball mill in the absence of solvent (Rodríguez et al., 2006; Hernández and Juaristi, 2011). Recently, there has been renewed interest in replacing complex catalytic systems with simpler amino acids. It was demonstrated that the use of amino acids in a water-methanol solvent mixture maintains high stereoselectivity of the process and reduces the reaction time to just four hours while achieving the yield of up to 84% (Emma et al., 2020).

This process has been successfully studied under microreactor conditions, however, the variety of the catalysts was very limited. For example, microreactors with a compact layer of silica functionalized with *L*-proline (L-Pro) were used for continuous reaction of **1** with **2** (Bortolini et al., 2012), leading to low yields and poor diastereoselectivity. The best results were attained using a derivative of L-Pro immobilized in polystyrene as a catalyst, containing a triazole linker (Sánchez-Antonio and Juaristi, 2019; Sánchez-Antonio et al., 2020). The Optimal reaction conditions in terms of yield, selectivity and recyclability were achieved in the 1: 1 DMF/H_2O mixture, but the condensation required long time to proceed due to the poor solubility of the substrates (Greco et al., 2016). Addition of ethanol to completely dissolve **1** and **2** resulted in decreased enantioselectivity towards the main *anti*-stereomer (<90% *ee*) and in noticeably reduced diastereoselectivity (*anti / syn* = 5: 1). Contrary to previously reported favourable effect of temperature on the activity of pyrrolidinyl tetrazole organocatalysts, performing the reaction at 50 °C resulted in a further enantioselectivity decrease (82% *ee*). The best diastereoselectivity was achieved using polystyrene-immobilized proline derivatives (Ayats et al., 2012). Regrettably, the authors reached good conversion and product yields only with prolonged reaction times (usually 24 h) in environmentally hazardous solvents. Rising the temperature resulted in a significant stereoselectivity decrease. Therefore, it became essential to find a new effective catalyst that would allow carrying the reaction out a) under environmentally acceptable conditions; b) with high conversion; c) over a short time while the reverse aldol reaction process has no significant negative effect on the process stereoselectivity.

The purpose of this work is to develop an approach to stereoselective synthesis under mild and environmentally friendly conditions in a microfluidic system using a set of amino acids as organocatalysts. We have optimized the process of 2-(hydroxy(4-nitrophenyl)methyl)cyclohexanone **3** synthesis using the sterically hindered non-protein amino acid L-*tert*-leucine (L-*tert*-Leu) (Bommarius et al., 1995). This process was selected as it is a popular model reaction under classical conditions (Guillena et al., 2008; Li et al., 2009; Xu et al., 2009; Mitsui and Parquette, 2009; Seebach et al., 2007; Zotova et al., 2009; Sharma and Sunoj, 2010; Orlandi et al., 2016; Renzi et al., 2017; Notz and List, 2000; List et al., 2001). Also, its final product **3** has various applications and exhibits a number of valuable properties. For example, it may be used as an effective cadmium marker (Sadia et al., 2017).

2. Material and methods

2.1. Instruments and materials

The synthesis was carried out in a flask or in a Qmix continuous flow microreactor produced by Wingflow AG, Switzerland. (Supporting information: Fig. S1). A metal T-shaped mixer and a 1000 mm stainless-steel coil with 1 mm internal diameter were used for the reaction. Quantitative analysis was performed on a Millikhrom A-02 HPLC instrument with a UV detector, using a ProntoSIL-120–5-C18 2 × 75 mm column with 5 mkm particle size. The analysis was performed with aqueous acetonitrile (40–80% acetonitrile) gradient elution at a flow rate of 200 mkl/min. Enantiomeric purity was monitored by HPLC on a chiral column (Chirobitic TAG column 250 × 4.6 mm; eluent: MeCN/C_6H_{12} (60/40) + 0.2% DEA; 0.70 mL/min, UV detector 210 nm) on an Agilent chromatograph. ^1H and ^{13}C NMR spectra were recorded on Agilent 400-MR and Bruker Avance-300 spectrometers, respectively, with working frequencies of 400 MHz and 300 MHz. Chemical shifts were reported in δ (ppm) relative to TMS as an internal standard, and coupling constants were reported in Hz. The solvent used was $CDCl_3$. The reaction progress was monitored by TLC on DC Kieselgel 60 F254 plates (MERCK).

The synthesized compounds were further purified by column chromatography on silica gel 60 (0.063–0.2 mm) from Macherey-Nagel, and their spectral characteristics corresponded to the literature data (Liu et al., 2014; Cobb et al., 2005). *p*-Nitrobenzaldehyde, cyclohexanone, ethanol, D,L-Pro, L-Pro, D-Val, L-*tert*-Leu, and L-His were obtained from AO ECOS-1 and SIGMA-Aldrich.

2.2. General synthetic methods

2.2.1. Method 1. Batch reaction

Modified from (Córdova et al., 2006; Rodríguez et al., 2006). *p-Nitrobenzaldehyde* (0.302 g, 2 mmol) and L-Pro (0.023 g, 0.2 mmol) were placed in a round-bottom flask with 10 mL of cyclohexanone. The reaction was carried out with constant stirring on a magnetic stirrer for 24 h. The mixture was then dried over anhydrous magnesium sulfate and concentrated under reduced pressure. 2-(Hydroxy(4-nitrophenyl) methyl)cyclohexanone **3** was isolated by column chromatography with ethyl acetate-hexane (1: 2 ratio) as the eluent with a gradient up to 1: 1. The conversion and diastereomeric ratio (*dr*) were determined by ^1H NMR spectroscopy. The enantiomeric excess (*ee*) for the individual stereoisomers was determined by chiral HPLC of the reaction mixture both

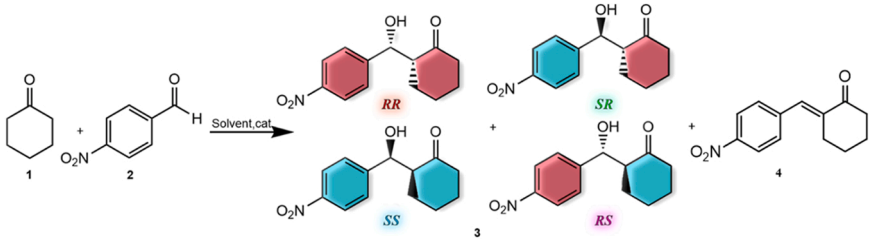

Fig. 1. Aldol reaction for the formation of 4 stereoisomers of 2-(hydroxyl (4-nitrophenyl)methyl)cyclohexanone **3** and product **4**.

before and after purification by flash column chromatography on silica gel.

2.2.2. Method 2. Microflow continuous process

2.2.2.1. Experimental setup for the continuous process. The reactions were carried out in a Qmixm microflow system by Wingflow AG. The system was modified by an external jacket filled with ethylene glycol in order to conduct the reaction at low temperatures (See Fig. 2). The reactor inlet was connected to syringe pumps, for continuous feed of both reactant solutions into the reactor. The outflow from the reactor was connected to a receiver flask that collected the product. Under these conditions, the dead volume was found to be 1.05 mL. The conversion and diastereoselectivity of the resulting product were determined by ^{1}H NMR spectroscopy of the periodically acquired samples. After the experiment, the solvent was removed under reduced pressure and the resulting aldol products were purified using flash chromatography on silica gel (hexane/ethyl acetate 4: 1–2: 1) after analysis.

The first syringe pump injected a 0.03 g/mL solution of *para*-nitrobenzaldehyde in aqueous ethanol, while the second pump delivered a solution containing 0.20 g/mL cyclohexanone and 0.0024 g/mL amino acid in the same solvent. Both solutions were fed into a stainless-steel T-mixer. A reaction column with a length of 1 m and a capillary diameter of 0.8 mm was placed after the T-mixer. The temperature of the reaction mixture was controlled by external heating. The interaction time was set each time by varying the reagent solutions flow rate.

2.2.2.2. Synthesis under microflow conditions using L-proline. Solutions **A** and **B** preparation. Solution **A**: 0.023 g (0.2 mmol) of L-proline was placed in a flask and dissolved in 8 mL of aqueous EtOH, followed by the addition of 2 mL (19.2 mmol) of cyclohexanone. Solution **B**: 0.302 g (2 mmol) of *p*-nitrobenzaldehyde was placed in a flask and dissolved in 10 mL of aqueous EtOH. The prepared solutions are transferred to syringes "**A**" and "**B**," respectively, and connected to the microreactor intake tubes. A T-shaped mixer and a 1000 mm long coil with an internal diameter of 1 mm were installed on the microfluidic reactor. The coil was heated uniformly. The reaction was initiated by turning on both syringe pumps. The flow rate was varied within the range of 0.083–1.0 mL/min. The feed speed rates were selected to keep the reagent ratio (p-nitrobenzaldehyde:cyclohexanone) at 1:10. The reaction temperature varied between – 10 and + 75 °C. The sample volume was up to 10 mL. The first sample was collected after 3 mL of the solution passed through the reactor. The reagent flow continued for 30 min, then the solvents were removed from the collected samples under reduced pressure providing a yellow oil. This oil was subjected to flash chromatography on silica gel (hexane / ethyl acetate = 2: 1) to obtain the target product **3** (4.87 g, 19.54 mmol, *dr* = 96: 4, 92% yield) as a white solid. The samples were analysed by NMR and HPLC before and after chromatography (Liu et al., 2014; Cobb et al., 2005).

The reactions with other amino acids as catalysts were carried out in the same manner.

3. Results and discussion

Optimization of the selected aldol condensation was carried out under microfluidic conditions, and compared with conventional conditions in a flask. In order to compare the results of the aldol condensation of ketone **1** with benzaldehyde **2** in a flask (Table 1, exp. 1 and 2) and under continuous conditions in a reactor (Fig. 2), the amino acid L-Pro was used (Table 1, exp. 4 – 11), which has been shown previously to exhibit good performance under classical reaction conditions (Samanta et al., 2005; Guizzetti et al., 2006; Qian et al., 2010; Kochetkov et al., 2011; Karmakar et al., 2011; Rodríguez et al., 2006; Hernández and Juaristi, 2011; Emma et al., 2020; Bortolini et al., 2012; Sánchez-Antonio and Juaristi, 2019; Sánchez-Antonio et al., 2020; Greco et al., 2016; Ayats et al., 2012). To find the best reaction conditions in the Qmix microreactor system, the following key parameters

Table 1
Aldol condensation reaction under microflow conditions in EtOH / H_2O catalyzed by Pro. *

Exp. #	Catalyst	T, °C	Time min	3, %	(*SR*)-3, *ee* %	anti/syn %
1**	D,L-Pro	25	24 h	89	–	7: 1
2**	L-Pro	25	24 h	90	92	9: 1
3	D,L-ProL	25	30	91	–	16:1
4	-Pro	25	30	96	90	14:1
5	L-ProL	10	30	95	92	14:1
6	-ProL	0	30	85	93	14:1
7	-ProL	−10	30	58	94	24:1
8	-ProL	25	5	26	83	6.5: 1
9	-ProL	25	10	46	87	13:1
10	-ProL	45	10	94	78	14:1
11	-Pro	75	30	83	75	10: 1

**Reaction in a flask.
* 10 mmol of **1** and 1 mmol of **2** were used, solvent - EtOH/H_2O (19:1), catalyst – 10 mol%, yield was determined by ^{1}H NMR with HPLC monitoring, Chirobitic TAG column 250 × 4.6 mm; 0.2% DEA, 0.70 mL min-1, 210 nm UV detector. "(*SR*)-**3**, ee %" means the enantioselectivity value of the main one of the stereoisomers relative to the opposite one, (*RS*)— **3**. "**3**, %" means the total yield of all stereoisomers of compound **3**.-

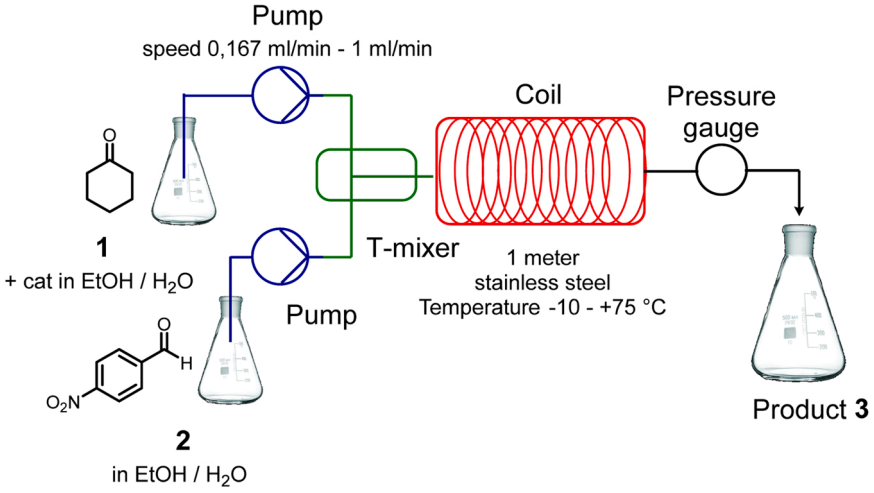

Fig. 2. Laboratory setup for aldol **3** synthesis in the Qmix microreactor.

were varied: flow rate, temperature, time, and catalyst concentration.

The reaction was carried out as follows. The first syringe pump injected a 0.03 g/mL solution of *para*-nitrobenzaldehyde in aqueous ethanol, while the second pump delivered a cyclohexanone 0.20 g/mL and amino acid 0.0024 g/mL solution in the same solvent. Both solutions were fed into a stainless steel T-mixer. After the mixer, a 1 m reaction column with internal diameter of 0.8 mm was installed. This allowed the temperature of the reaction mixture and the interaction time of the reagents to be changed by varying the reagents flow rate and the external heating.

Periodically collected reaction mass samples were analyzed by ^{1}H NMR spectroscopy and HPLC to determine the conversion and diastereoselectivity of the process. The enantiomeric purity of the product diastereomers was determined by chiral HPLC.

The individual reaction products were synthesized by the classical batch process (Córdova et al., 2006) (Table 1, exp.1, 2; Fig. 3d). The individual products of the condensation reaction: (*SR*)- and (*RS*)-stereoisomers of the major *anti*-diastereomer and (*SS*)- and (*RR*)-stereoisomers of a minor *syn*-diastereomer were isolated to be used as references. Among the reaction products, 2-(4-nitrobenzylidene)cyclohexanone **4** was isolated and characterized as an unsaturated side product of the target compound dehydration, the yield of **4** never exceeded 7%.

During the initial experiments in the microflow system, the reaction time and temperature were varied in the range of 15 min to 1 h and 10–75 °C respectively, in environmentally acceptable aqueous ethanol (Greco et al., 2016). L-Pro was used as a catalyst at a concentration of 5 – 10 mol%. It was found that the highest conversion of 99% was achieved at 25 °C and an amino acid concentration of 10 mol% after a 30-minute reaction in aqueous (5% water) ethanol. When microflow conditions were used, the yield increased compared to the classical conditions (Table 1, exp. 1, 2), and the reaction time was significantly reduced (Table 1, exp. 3, 4). Increasing the temperature from 25° to 75°C resulted in a slight increase (up to 5 – 7% by mass) of the dehydration product **4** and a decrease in enantioselectivity (Table 1, exp. 10, 11). It was determined that at a flow rate of less than 0.333 mL/min, which corresponds to a reactor residence time of more than 30 min, there was a decrease in conversion and consequently a reaction yield of up to 95%. The enantioselectivity of the L-Pro catalyzed process after 30 min at 25 °C was 90% for the predominantly formed (*SR*)-enantiomer of the main *anti*-diastereomer (syn/anti = 1: 8) (Table 1, Fig. 3c). The reaction time was reduced by almost an order of magnitude compared to classical conditions, with similar product yields (Córdova et al., 2006).

The significant process acceleration allowed us to conduct a series of experiments at lower temperatures, which naturally led to an increase in the enantioselectivity of the process (Fig. 3a-c). The enantioselectiviry for the main (*SR*)-stereoisomer of aldol **3** was 92% at 10 °C, 93% at 0 °C (Fig. 3b) and 94% at − 10 °C (Fig. 3a), respectively, with high values of *anti / syn* diastereoselectivity (Table 1, exp. 4 – 7). Moreover, the temperature increase to 45 and 75 °C led not only to a significant decrease in the enantioselectivity of the process (Fig. 4a; Table 1, exp. 10, 11), but also to an increase in the initial trace amounts of the dehydration product **4** (up to 7% by mass). A similar, although less pronounced, result of decreasing enantioselectivity by increasing the reaction temperature was obtained when using another amino acid, L-*tert*-Leu (Table 2, exp. 1 – 4, Fig. 4b).

Using the opposite configuration amino acid D-valine (D-Val) as an organocatalyst showed the expected high *ee* value of 96% for the other (*R,S*)-enantiomer of the same major *anti*-diastereomer **3**, which was also formed in significant excess compared to the *syn*-diastereomer **3** in a ratio of 16.5:1 (Fig. 5b, Table 2, exp. 6). It can be assumed that the higher enantioselectivity values compared to the same reaction conditions conducted in a flask were achieved by reducing the probability of the reverse aldol reaction (that leads to the racemization of the main reaction product) due to significant (by more than an order of magnitude) reduction of the reaction time. The slow racemization effect was observed earlier when the aldol reaction time was increased from 48 h (91% *ee*) to 120 h (83% *ee*) (Córdova et al., 2006) and it was associated with the reversibility of the aldol formation process. An additional advantage of microflow process setup is the reduction of the unsaturated side product **4** to trace levels. Transition to L-*tert*-Leu – a more sterically hindered amino acid than Val or Pro – led to a greater improvement in a range of indicators, in particular, stereospecificity and process rate. Indeed, when L-*tert*-Leu at 25 °C was used as a catalyst, the reaction was over in 15 min, and high diastereoselectivity (25:1) was achieved with the highest enantiomeric purity of 97% for the preferred (*SR*)-*anti*-stereomer of the target product **3** (Fig. 5c, Table 2, exp. 4). Conducting the process at lower temperatures, as in the case of L-Pro, leads to increased enantioselectivity of the process (Table 2, exp. 1 – 3). At the same time, using L-histidine (L-His) as a catalyst in the same amount (Fig. 5a, Table 2, exp. 7) not only reduces enantioselectivity but also severely reduces the diastereoselectivity of the process, which is consistent with known data (Córdova et al., 2006; Jiang et al., 2010) regarding this amino acid catalyst.

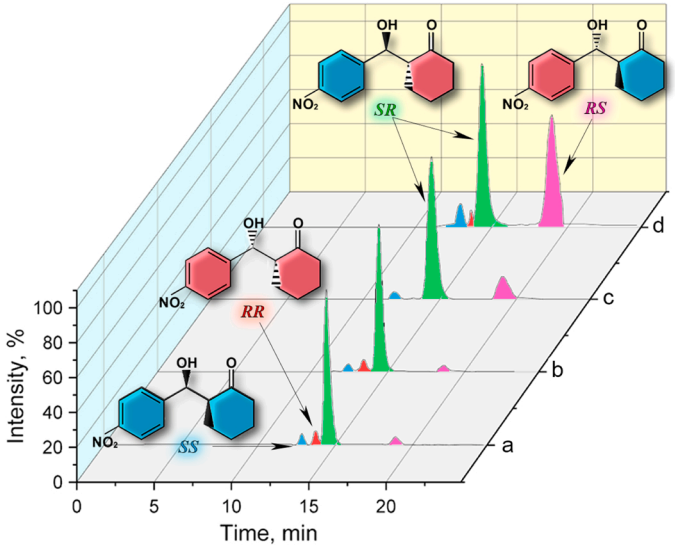

Fig. 3. Isomers of **3** formed with proline catalysis: a) L-Pro at − 10 °C (ee 94%, anti/syn 14:1), b) L-Pro at 0 °C (ee 93%, 14:1), c) L-Pro at 25 °C (ee 92%, 24:1), d) DL-Pro at 25 °C (ee 91%, 16:1) flow rate 0.33 mL/min.

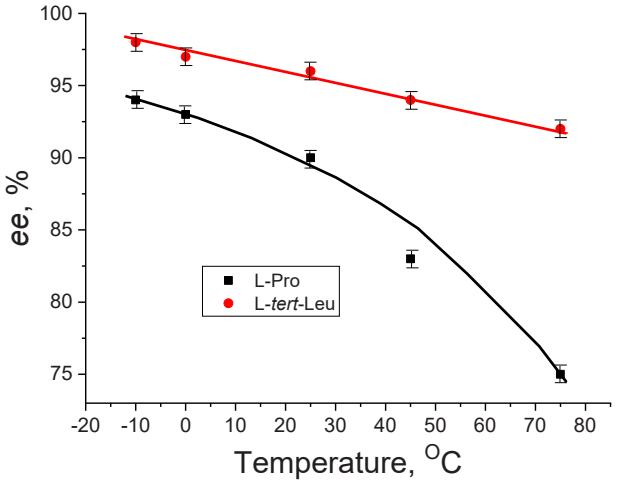

Fig. 4. Enantioselectivity of the formation of aldol **3** during the catalysis of 10 mol% L-Pro (a) and L-*tert*-Leu. (b) in a continuous flow in the microreactor depending on the process temperature from − 10 to + 75 °C.

Table 2
Aldol condensation reaction under microflow conditions in EtOH / H_2O catalyzed by amino acids*.

Nº	Amino acid	T, °C	Time min	3, %	(*SR*)– 3, *ee* %	anti /syn, %
1	L-*tert*-Leu	-10	30	85	98	28: 1
2	L-*tert*-Leu	0	30	90	97	26: 1
3	L-*tert*-Leu	45	15	93	94	23: 1
4	L-*tert*-Leu	25	15	99	96	25: 1
5	L-*tert*-Leu**	25	15	82	88	6: 1
6	D-Val	25	15	99	92 (*RS*-**3**)	9:1
7	L-His	25	20	90	54 (*RS*-**3**)	3:1

** Solvent – absolute ethanol.
[*] 10 mmol of **1** and 1 mol of **2** were used, solvent - EtOH/H_2O (19:1), catalyst – 10 mol%, yield was determined by ^{1}H NMR with HPLC monitoring, Chirobitic TAG column 250 × 4.6 mm; 0.2% DEA, 0.70 mL min-1, 210 nm UV detector.

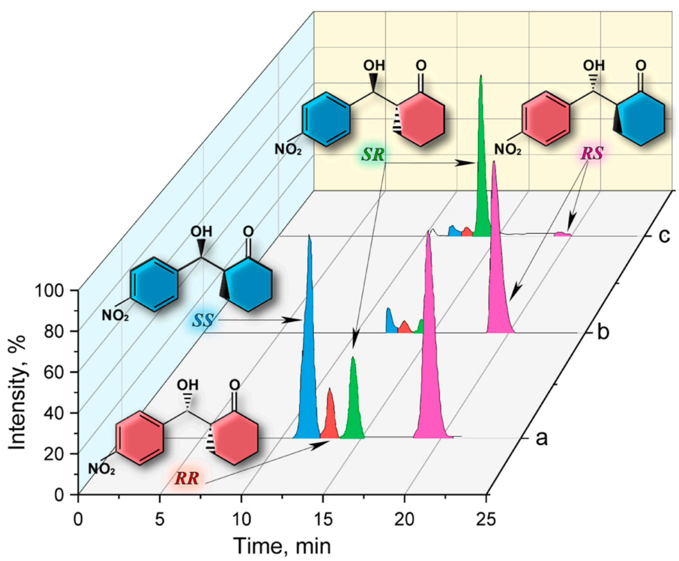

Fig. 5. Isomers of **3** formed with amino acid (10 mol%) catalysis: a) L-His (ee 54%, 3:1), b) D-Val (ee 92%, 9:1), c) L-*tert*-Leu (yield SS-product – 5,5; RR – 5,5; SR – 85; RS – 4 (ee 98%, 28:1)) flow rate 0.67 mL/min.

The stereochemistry of the main aldol product **3**, obtained via acyclic amino acid catalysis, was assigned to the *SR*-configuration, as determined by HPLC analysis on the chiral stationary phase and comparison with literature data (Guillena et al., 2008; Li et al., 2009; Bañón-Caballero et al., 2013; Córdova et al., 2006; Cho and Kim, 2014). The relative stereoisomery of the cyclic aldol products was assigned *anti*-, as determined by NMR analysis and correlated with literature sources (Guillena et al., 2008; Bañón-Caballero et al., 2013; Cho and Kim, 2014). Based on the relative and absolute stereochemistry of the aldol products, a chair-like six-membered cyclic transition states **I** and **II** (Fig. 6a, b) can be proposed to explain the stereochemical results of the reactions catalysed by acyclic amino acids L-Pro and L-*tert*-Leu, respectively. The more rigidly shaped transition state **II** in the case of catalysis by L-*tert*-Leu (Fig. 6b) leads to higher enantioselectivity values. According to the proposed scheme, the *re*-face of the acceptor aldehyde undergoes attack by the *si*-face of the chiral enamine, forming the *anti*-aldol product. This transition state is also in agreement with density functional theory (DFT) calculations for aldol reactions catalysed by alanine (Dondoni and Massi, 2008; Zlotin et al., 2009; Bassan et al., 2005; Clemente and Houk, 2005; Armstrong et al., 2014).

In addition, we have shown that the proton transfer from the carboxyl group of the amino acid and the activation of hydrogen bonding promoted by the presence of a small amount of water (5%) are crucial for the strong asymmetric induction (Table 2, exp. 5). The superior enantioselectivity found in the reactions with cyclic ketone **1**, catalysed by amino acids, can be attributed to the transition states **I** and **II** stabilization due to the rigidity of the cyclohexane ring. In contrast, the significant increase of the α-substituent volume in the amino acid, as proline was replaced with *tert*-leucine, (i.e., the change from **I** to **II**) also limits the possibility of other transition states that lead to the formation of alternative stereoisomers. Using *tert*-leucine to catalyse the assembly of the product with high enantioselectivity, undoubtedly maintains the ordered transition state **II** in the aqueous environment.

For proline-catalysed reactions, enantioselectivity is increased when water is present (Table 2, exp. 4, 5). A small amount of water likely improves the enantioselectivity by increasing the Brønsted acidity of the acyclic amino acid. Further increase in the amount of water (>10% by volume) decreases the enantioselectivity of the aldol reaction, confirming the hydrogen bonding as an essential feature of transition states **I** and **II**. All these results demonstrate the fact that the hydrogen bond from the acidic fragment is crucial in the transition state, making possible to form a highly ordered transition state in aqueous conditions.

4. Conclusion

The microfluidic approach was vital to the development of the organocatalytic asymmetric aldol condensation of cyclohexanone **1** with *p*-nitrobenzaldehyde **2** in the aqueous ethanol medium in the presence of 10 mol% L-*tert*-Leu to produce the desired β-hydroxyketone **3** with a nearly quantitative yield and diastereoselectivity of up to 96% and enantiomeric purity of the major stereoisomer up to 98%. This means that essentially only one key *SR*-stereoisomer was formed in the reaction. Comparison of the two experimental methods for aldol condensation under classical batch conditions (in a flask) and in a microflow system demonstrated the advantages of the microfluidic method, which provides comparable product yield and stereoselectivity while reducing the reaction time by an order of magnitude. This fact allowed to carry

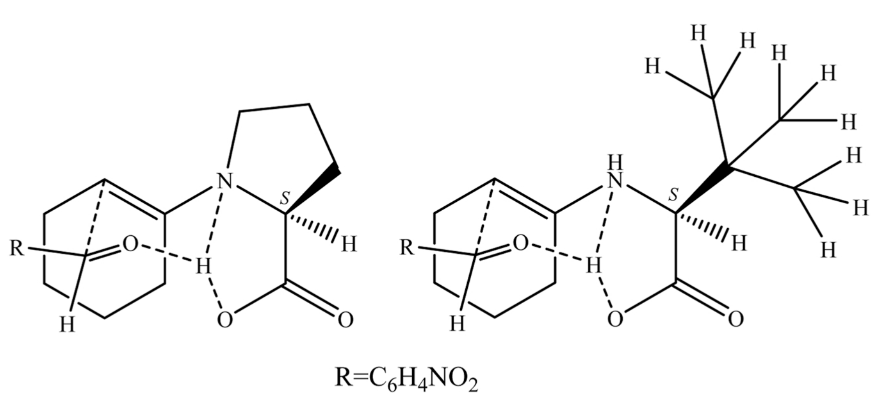

Fig. 6. Transition states for aldol **3** formation catalyzed by a) L-proline (**I**) and b) L-*tert*-leucine (**II**).

out the reaction at a lower temperature (−10 °C) with even higher enantio- and diastereoselectivity in 30 min with high conversion. The reaction mechanism was suggested to explain the regularities in the observed values of stereoselectivity. Moreover, our study demonstrated that easily available amino acids have the potential to be used in direct asymmetric aldol reactions as highly selective organocatalysts. Thus, the entire diversity of the natural structures can be used in the development and combinatorial synthesis of non-toxic and straightforward organic catalysts.

CRediT authorship contribution statement

Konstantin A. Kochetkov: Writing – original draft, Methodology, Resources. **Maxim S. Oshchepkov:** Project administration, Funding acquisition, Validation. **Pavel A. Pavlov:** Investigation, Writing – original draft. **Michail M. Il'in:** Formal analysis, Investigation, Data curation. **Inna N. Soloveva:** Validation, Formal analysis. **Alexander S. Oshchepkov:** Visualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The work was carried out within the framework of the State Task No. 075-03-2023-642 of the Ministry of Science and Higher Education of the Russian Federation using the scientific equipment of the Institute of Structural Molecules Research of the RAS and the Russian Science Foundation grant No. 22-73-10076 (2022).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cherd.2023.11.055.

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